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FILE COVERS 1907 - 8 Oct 2002  VOL 137 ISS 15
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the CAS Roles thesaurus (/RL field) in this file.

=> d stat que
L1             2 SEA FILE=REGISTRY ("VACCINIA VIRUS GROWTH FACTOR (VACCINIA
                 VIRUS)"/CN OR "VACCINIA VIRUS PROTEINASE"/CN)
L2            12 SEA FILE=REGISTRY HEPATITIS C CORE ANTIGEN?/CN
L4          4408 SEA FILE=REGISTRY ENVELOPE PROTEIN?/CN
L9          7320 SEA FILE=HCAPLUS L1 OR VACCINIA(W)VIR?
L10         7780 SEA FILE=HCAPLUS L2 OR HEPATITIS(W)C(W)VIR? OR HCV
L11         8502 SEA FILE=HCAPLUS L4 OR ENVELOPE?(W)PROTEIN?
L12          307 SEA FILE=HCAPLUS L10 (L)L11
L13           77 SEA FILE=HCAPLUS L12 AND (RECOMBINANT OR L9)
L14           32 SEA FILE=HCAPLUS L13 AND (PURIF? OR PRODUCT? OR MANUF?)
L15           15 SEA FILE=HCAPLUS L14 AND VACCIN?


=> d ibib abs hitrn l15 1-15

L15  ANSWER 1 OF 15  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2002:539704  HCAPLUS
DOCUMENT NUMBER:          137:108289
TITLE:                    Purified hepatitis C virus envelope E1
                          and/or E2 proteins for diagnostic and therapeutic use
INVENTOR(S):              Maertens, Geert; Bosman, Fons; Buyse, Marie-Ange
PATENT ASSIGNEE(S):       Innogenetics N.V., Belg.
SOURCE:                   PCT Int. Appl., 243 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
```

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002055548	A2	20020718	WO 2002-EP219	20020111

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-315768P P 20010830

AB The present invention relates to a method for **purifying recombinant HCV** single or specific oligomeric **envelope proteins** selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or redn. step is carried out with a disulfide bond cleavage agent. The present invention also relates to a compn. isolated by such a method. The present invention also relates to the diagnostic ad therapeutic application of these compns. Furthermore, the invention relates to the use of **HCV** E1 protein and peptides for prognosing and monitoring the clin. effectiveness and/or clin. outcome if **HCV** treatment.

L15 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:471385 HCAPLUS
DOCUMENT NUMBER: 137:197234
TITLE: Reconstitution of hepatitis C virus envelope glycoproteins into liposomes as a surrogate model to study virus attachment
AUTHOR(S): Lambot, Michel; Fretier, Stephanie; Op De Beeck, Anne; Quatannens, Brigitte; Lestavel, Sophie; Clavey, Veronique; Dubuisson, Jean
CORPORATE SOURCE: CNRS-Institut de Biologie de Lille and Institut Pasteur de Lille, Lille, 59021, Fr.
SOURCE: Journal of Biological Chemistry (2002), 277(23), 20625-20630
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The envelope glycoproteins, E1 and E2, of hepatitis C virus (HCV) assemble intracellularly to form a noncovalent heterodimer that is expected to be essential for viral assembly and entry. However, due to the lack of a cell culture system supporting efficient HCV replication, it is very difficult to obtain relevant information on the functions of this glycoprotein oligomer. To get better insights into its biol. and biochem. properties, HCV envelope glycoprotein heterodimer expressed by a

vaccinia virus recombinant was **purified** by immunoaffinity. **Purified** E1E2 heterodimer was recognized by conformation-dependent monoclonal antibodies, showing that the proteins were properly folded. In addn., it interacted with human CD81, a putative HCV receptor, as well as with human low and very low d. lipoproteins, which have been shown to be assocd. with infectious HCV particles isolated from patients. **Purified** E1E2 heterodimer was also reconstituted into liposomes. E1E2-liposomes were recognized by a conformation-dependent monoclonal antibody as well as by human CD81. Together, these data indicate that E1E2-liposomes are a valuable tool to study the mol. requirements for HCV binding to target cells.

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:43928 HCAPLUS
DOCUMENT NUMBER: 136:277718
TITLE: Live and Killed Rhabdovirus-Based Vectors as Potential Hepatitis C **Vaccines**
AUTHOR(S): Siler, Catherine A.; McGettigan, James P.; Dietzschold, Bernhard; Herrine, Steven K.; Dubuisson, Jean; Pomerantz, Roger J.; Schnell, Matthias J.
CORPORATE SOURCE: The Dorrance H. Hamilton Laboratories, Center for Human Virology, Departments of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA, 19107, USA
SOURCE: Virology (2002), 292(1), 24-34
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A highly attenuated, **recombinant** rabies virus (RV) **vaccine** strain-based vector was utilized as a new immunization strategy to induce humoral and cellular responses against hepatitis C (**HCV**) glycoprotein E2. The authors showed previously that RV-based vectors are able to induce strong immune responses against human immunodeficiency virus type 1 (HIV-1) antigens. Here they constructed and characterized 3 replication-competent RV-based vectors expressing either both **HCV envelope proteins** E1 and E2 or a modified version of E2 which lacks 85 amino acids of its C terminus and contains the human CD4 transmembrane domain and the CD4 or RV glycoprotein cytoplasmic domain. All 3 constructs stably expressed the resp. protein(s) as indicated by Western blotting and immunostaining. Moreover, surface expression of **HCV** E2 resulted in efficient incorporation of the **HCV envelope protein** regardless of the presence of the RV G cytoplasmic domain, which was described previously as a requirement for incorporation of foreign glycoproteins into RV particles. Killed and **purified** RV virions contg. **HCV** E2 were highly immunogenic in mice and also proved useful as a diagnostic tool, as indicated by a specific reaction with sera from **HCV**-infected patients. In addn., RV **vaccine** vehicles were able to induce cellular responses against **HCV** E2. Thus, **recombinant** RVs are potentially useful **vaccine** vectors against important human viral diseases. (c) 2002 Academic Press.

REFERENCE COUNT: 61 THERE ARE 61 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:912918 HCAPLUS
DOCUMENT NUMBER: 137:150837
TITLE: Effect of downstream sequence on the cleavage of envelop protein 1 signal sequence in Hepatitis C virus
AUTHOR(S): Zhu, Lixin; Kong, Yuying; Wang, Yuan; Li, Guangdi
CORPORATE SOURCE: Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(6), 682-686
CODEN: SHWPAU; ISSN: 0582-9879
PUBLISHER: Shanghai Kexue Jishu Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The RNA genome of hepatitis C virus encodes a polyprotein of about 3,000 amino acids, which is processed into 10 viral protein by proteases provided by host cells and virus itself, multiple precursors are produced due to inefficient processing. E1 signal sequence (C/E1 site) processing in eukaryotic **vaccinia virus**/T7 system was studied. Differently truncated HCV structural proteins were expressed in this system. It was found that the efficient cleavage of E1 signal sequence was affected by downstream envelop protein sequences. When the lacZ gene encoding a **product** with similar size was engineered downstream to the E1 signal sequence, the inefficient cleavage of signal sequence was also obsd., suggesting that the effect of downstream sequence on the cleavage was due to the presence of the envelop protein sequences. Computer-aided anal. clearly showed that E1 signal sequences was a typical signal sequence. The influence of downstream sequences to signal sequence cleavage demonstrated here was uncommon. To date, similar observations were only reported for the processing of IL-12 signal sequence and the C/prM site of flavivirus.

L15 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:467599 HCAPLUS
DOCUMENT NUMBER: 129:199513
TITLE: Characterization of the structural proteins of hepatitis C virus expressed by an adenovirus **recombinant**
AUTHOR(S): Rim Seong, Young; Lee, Chan-Hee; Im, Dong-Soo
CORPORATE SOURCE: Gene Therapy Research Unit, Korea Research Institute of Bioscience and Biotechnology, Taejeon, S. Korea
SOURCE: Virus Research (1998), 55(2), 177-185
CODEN: VIREDF; ISSN: 0168-1702
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human adenoviruses have been used for mammalian expression vectors and **recombinant vaccines** for heterologous antigens. The authors constructed and characterized an infectious adenovirus **recombinant** contg. core-E1-E2 genes of **hepatitis**

C virus (**HCV**). The core protein was produced mainly during the early phase of viral infection. Expression of **HCV** E1 and E2 **envelope proteins** was detected by an immunopptn. with **HCV**-pos. patient's sera. The **purified** E1 and E2 proteins appeared to be composed of mainly a heterodimeric form via noncovalent interaction, as previously obsd. in other mammalian expression systems. A small portion of E1 and E2 monomers as well as E1E2 aggregates by inter-disulfide linkage were detected. Apparently heterodimeric E1E2 complexes were serol. reactive. The results suggest that adenovirus is an useful **HCV** antigen-expression vector.

L15 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:344513 HCAPLUS
DOCUMENT NUMBER: 129:24164
TITLE: Synthesis and **purification** of hepatitis C virus-like particles from insect cells using a baculovirus vector
INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; Liang, T. Jake; Baumert, Thomas F.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325
US 6387662	B1	20020514	US 1999-296441	19990421

PRIORITY APPLN. INFO.: US 1996-30238P P 19961108
WO 1997-US5096 W 19970325

AB Prodn. of enveloped RNA virus-like particles intracellularly in vitro in insect cells using a **recombinant** baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and **purifn**. of hepatitis C virus (HCV)-like particles contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the **purified** HCV-like particles is

disclosed.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:266203 HCAPLUS
DOCUMENT NUMBER: 129:14336
TITLE: Hepatitis C virus structural proteins assemble into viruslike particles in insect cells
AUTHOR(S): Baumert, Thomas F.; Ito, Susumu; Wong, David T.; Liang, T. Jake
CORPORATE SOURCE: Liver Diseases Section, National Institute Diabetes and Digestive and Kidney Diseases, National Institute Health, Bethesda, MD, 20892, USA
SOURCE: Journal of Virology (1998), 72(5), 3827-3836
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Hepatitis C virus** (**HCV**) is a leading cause of chronic hepatitis in the world. The study of **HCV** has been hampered by the low level of viral particles in infected individuals, the inability to propagate efficiently the virus in cultured cells, and the lack of a convenient animal model. Due to these obstacles, neither the structure of the virus nor the prerequisites for its assembly have been clearly defined. Here, the authors describe a model for the prodn. and **purifn**. of **HCV**-like particles in insect cells using a **recombinant** baculovirus contg. the cDNA of the **HCV** structural proteins. In insect cells, expressed **HCV** structural proteins assembled into enveloped viruslike particles (40 to 60 nm in diam.) in large cytoplasmic cisternae, presumably derived from the endoplasmic reticulum. Biophys. characterization of viruslike particles by CsCl and sucrose gradient centrifugation revealed biophys. properties similar to those of putative virions isolated from infected humans. The results suggested that **HCV** core and **envelope proteins** without p7 were sufficient for viral particle formation. Anal. of particle-assocd. nucleic acids demonstrated that **HCV** RNAs were selectively incorporated into the particles over non-**HCV** transcripts. The synthesis of **HCV**-like particles in insect cells may provide an important tool to det. the structural requirements for **HCV** particle assembly as well as to study viral genome encapsidation and virus-host interactions. The described system may also represent a potential approach toward **vaccine** development.

L15 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:205500 HCAPLUS
DOCUMENT NUMBER: 128:290843
TITLE: Expression of structural proteins of hepatitis C virus (HCV) in mammalian cells
AUTHOR(S): Li, Yingchun; Li, Guangdi; Kong, Yuying; Wang, Yuan; Wang, Yu; Wen, Yumei
CORPORATE SOURCE: Shanghai Inst. Biochemistry, Chinese Academy Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Science in China, Series C: Life Sciences (1998),

41(1), 47-55
CODEN: SCCLFO; ISSN: 1006-9305
PUBLISHER: Science in China Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **vaccinia viral** vector contg. T7 promoter was used to construct the expression plasmids carrying **HCV** structural genes of C, E1 and E2/NS1. These genes were transiently expressed in mammalian cells in the presence of the T7 RNA polymerase which was provided by the **recombinant vaccinia virus** vTT7. Expression of mature core protein, **envelope protein** E1 and E2 was detected by Western blot using **HCV** patient sera as the primary antibodies. It was found that the sera from different **HCV** patients reacted differently with the expressed **products**, so did the sera collected at different times from the same patient, from whom the **HCV** structural genes were isolated. Among six mammalian cell lines, Vero and HeLa were the most suitable for the expression of C, E1 and E2. The **recombinant vaccinia viruses** have been constructed to constantly produce the C, E1 and E2 proteins for further research.

L15 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:181141 HCAPLUS
DOCUMENT NUMBER: 126:170383
TITLE: **Hepatitis C virus vaccines** comprising an oil-in-water emulsion containing QS21, deacylated monophosphoryl lipid A, and viral core protein or **envelope protein**
INVENTOR(S): Cabezon, Silva Teresa; Momin, Patricia Marie; Garcon, Nathalie Marie-Josephe
PATENT ASSIGNEE(S): Smithkline Beecham Biologicals S.A., Belg.; Cabezon Silva Teresa; Momin, Patricia Marie; Garcon, Nathalie Marie-Josephe Claude
SOURCE: PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9701640	A2	19970116	WO 1996-EP2764	19960620
WO 9701640	A3	19970515		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
CA 2222456	AA	19970116	CA 1996-2222456	19960620
AU 9663049	A1	19970130	AU 1996-63049	19960620
EP 835318	A2	19980415	EP 1996-922029	19960620

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI

CN 1189855	A	19980805	CN 1996-195150	19960620
BR 9609258	A	19990511	BR 1996-9258	19960620
JP 11508769	T2	19990803	JP 1996-504167	19960620
ZA 9605459	A	19970401	ZA 1996-5459	19960627
NO 9706060	A	19980217	NO 1997-6060	19971223

PRIORITY APPLN. INFO.: GB 1995-13261 A 19950629
WO 1996-EP2764 W 19960620

AB A **vaccine** compn. comprises QS21; 3 de-O-acylated monophosphoryl lipid A (3D-MPL); an oil in water emulsion contg. a metabolizable oil, such as squalene, alpha tocopherol and Tween 80;, and at least one immunogen selected from the group consisting of (a) a **hepatitis C virus** core protein or an immunogenic deriv. thereof, and (b) a **hepatitis C virus envelope protein** or an immunogenic deriv. thereof. A fusion protein contg. influenza virus NS1 protein fragment fused to **hepatitis C virus** core protein was prepd. with **recombinant** Escherichia coli. The **purified** fusion protein was formulated with QS21, 3D-MPL, and an oil-in-water emulsion contg. squalene, tocopherol, and Tween 80.

L15 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:54036 HCAPLUS
DOCUMENT NUMBER: 126:73782
TITLE: Unprocessed core-envelope fusion protein and nonstructural protein for the diagnosis of and **vaccination** against hepatitis C virus
INVENTOR(S): Liao, Jaw-Ching; Wang, Cheng-Nan
PATENT ASSIGNEE(S): Bionova Corporation, USA; Liao, Jaw-Ching; Wang, Cheng-Nan
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9637606	A1	19961128	WO 1996-US7378	19960522

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI

RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN

ZA 9604094	A	19961203	ZA 1996-4094	19960522
AU 9659243	A1	19961211	AU 1996-59243	19960522

PRIORITY APPLN. INFO.: US 1995-447276 19950522
WO 1996-US7378 19960522

AB The unprocessed core protein region initially translated from the genome of hepatitis C virus (HCV) contains epitopic configurations that are not retained in the processed proteins. In particular, the core protein loses

an epitopic configuration upon processing at the cleavage site between the genomic region (e.g., gene) encoding the core protein and the genomic region encoding the adjacent envelope region. The unprocessed epitopic configuration of the core region provides an improved ability to detect the presence of HCV, or antibodies to HCV, in a sample, including an unpurified sample or a sample of very small vol. (which can be particularly helpful when testing a sample from an infant or other person having very little blood (or other suitable material) available for testing). Combining the unprocessed core region with a nonstructural protein (such as an NS5 or an NS3-NS4 fusion) results in a synergistic effect that greatly enhances the already improved sensitivity and specificity provided by the unprocessed core region. The unprocessed epitopic configuration of the core region also provides an improved ability to induce an immune response upon administration of the core region into an animal. **Recombinant** methods are described for the prepn. of a cloned DNA mol. (EN-80-2) derived from the HCV core and envelope regions and for a clone (EN-80-1) encoding the NS5 nonstructural protein.

L15 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:295079 HCAPLUS
DOCUMENT NUMBER: 124:352673
TITLE: **Recombinant production** and **purification** of **hepatitis C virus envelope proteins** for diagnostic and therapeutic use
INVENTOR(S): Maertens, Geert; Bosman, Fons; De Martynoff, Guy; Buyse, Marie-Ange
PATENT ASSIGNEE(S): Innogenetics N.V., Belg.
SOURCE: PCT Int. Appl., 146 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9604385	A2	19960215	WO 1995-EP3031	19950731
WO 9604385	A3	19960307		
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2172273	AA	19960215	CA 1995-2172273	19950731
AU 9533824	A1	19960304	AU 1995-33824	19950731
AU 708174	B2	19990729		
EP 721505	A1	19960717	EP 1995-930434	19950731
EP 721505	B1	20020508		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09503396	T2	19970408	JP 1995-506189	19950731

BR 9506059	A	19971028	BR 1995-6059	19950731
AT 217345	E	20020515	AT 1995-930434	19950731
EP 1211315	A1	20020605	EP 2002-3643	19950731
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
US 6150134	A	20001121	US 1996-612973	19960311
US 6245503	B1	20010612	US 1997-927597	19970911
PRIORITY APPLN. INFO.:			EP 1994-870132 A	19940729
			EP 1995-930434 A3	19950731
			WO 1995-EP3031 W	19950731
			US 1996-612973 A3	19960311

AB **Envelope proteins** E1 and E2 of **hepatitis C virus** (**HCV**), their **recombinant** prodn. and **purifn.**, their fragments and engineered derivs., their antigenic epitope peptides, their monoclonal antibodies, and their use for diagnostic and therapeutic means are provided. A method is described for **purifying recombinant HCV** single or specific oligomeric **envelope proteins**, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulfide bond cleavage or redn. step is carried out with a disulfide bond cleavage agent (such as dithiothreitol and/or Empigen BB) and an SH group protecting agent (such as N-ethylmaleimide). Various forms of the E1 and E2 proteins are constructed by std. genetic techniques using **vaccinia virus** recombination vectors; such proteins are specific for various **HCV** genotypes, may delete the hydrophobic region from E1, or remove various glycosylation sites; they may also add factor Xa cleavage sites and His6 tags for improved **purifn.** Epitope (such as F, G, H, and I) peptides are used to generate monoclonal antibodies and to monitor disease progression in patients. Furthermore, the **HCV** E1 protein and peptides are used for prognosing and monitoring the clin. effectiveness and/or clin. outcome of **HCV** treatment.

L15 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	1996:42631 HCAPLUS
DOCUMENT NUMBER:	124:84303
TITLE:	High efficiency prokaryotic expression and **purification** of a portion of the hepatitis C core protein and analysis of the immune response to **recombinant** protein in BALB/c mice
AUTHOR(S):	Hitomi, Y.; McDonnell, W. M.; Baker, J. R., Jr.; Askari, F. K.
CORPORATE SOURCE:	Dep. Internal Medicine, Univ. Michigan, Ann Arbor, MI, 48109-0680, USA
SOURCE:	Viral Immunology (1995), 8(2), 109-19 CODEN: VIIMET; ISSN: 0882-8245
PUBLISHER:	Liebert
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB **Hepatitis C virus** (**HCV**) produces chronic persistent liver infection in 1-2% of the U.S. population and is the leading cause of end stage liver disease in patients presenting for liver transplantation at our center. Efforts to cure persistent **HCV** infection are frequently unsuccessful, so the development of a **HCV vaccine** is a high priority. **HCV**

envelope proteins are hypervariable so prodn. of a **recombinant** surface antigen **vaccine** such as is available for hepatitis B is not likely to confer widespread, high level protective immunity. As the most highly conserved structural protein in the **HCV** genome, the core protein is one reasonable target for **vaccine** prodn. Presented here are data on the **manuf.** of **recombinant** core protein contg. partial carboxy terminus deletions in an effort to increase the efficiency of core expression. The maltose binding protein (MBP) and glutathione S-transferase (GST) protein prokaryotic expression systems were used to study two different constructs, expressing the first 140 and 163 amino acids of the core region. Deletion of the 23 amino acids (aa) from aa141-163 led to a marked increase in the efficiency of protein prodn. from <1 to 3-4 mg/L for both systems studied. Protein **purifn.** was accomplished using affinity chromatog. (MBP) or inclusion body isolation (GST) as detd. by SDS-PAGE gels and immunotransblot with **HCV** core protein-specific monoclonal antibody. Finally, the immune response to **recombinant** protein was assessed in BALB/c mice using a MBP **HCV** core fusion protein and an ELISA developed using GST **HCV** core protein as a target. In all mice of this strain, serum anti-**HCV** core antibody titer increased to 10-4, two logs above background, following immunization in conjunction with Freund's complete adjuvant. These results represent an encouraging first step toward prodn. of a core protein **vaccine**. **Recombinant** core protein is a useful tool to study the immune response to core protein and may be useful to further study the epidemiol. and biol. of the **HCV** virus.

L15 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:4188 HCAPLUS
DOCUMENT NUMBER: 120:4188
TITLE: Characterization of hepatitis C virus envelope glycoprotein complexes expressed by **recombinant vaccinia viruses**
AUTHOR(S): Ralston, Robert; Thudium, Kent; Berger, Kim; Kuo, Carol; Gervase, Barbara; Hall, John; Selby, Mark; Kuo, George; Houghton, Michael; Choo, Qui Lim
CORPORATE SOURCE: Chiron Corp., Emeryville, CA, 94608, USA
SOURCE: Journal of Virology (1993), 67(11), 6753-61
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors constructed **recombinant vaccinia virus** vectors for expression of the structural region of **hepatitis C virus** (**HCV**). Infection of mammalian cells with a vector (vv/HCV1-906) encoding C-E1-E2-NS2 generated major protein species of 22 kDa (C), 33 to 35 kDa (E1), and 70 to 72 kDa (E2), as obsd. previously with other mammalian expression systems. The bulk of the E1 and E2 expressed by vv/HCV1-906 was integrated into endoplasmic reticulum membranes as core-glycosylated species, suggesting that these E1 and E2 species represent intracellular forms of the **HCV envelope proteins**. **HCV** E1 and E2 formed E1-E2 complexes which were pptd. by either anti-E1 or anti-E2 serum and which sedimented at approx. 15 S on glycerol

d. gradients. No evidence of intermol. disulfide bonding between E1 and E2 was detected. E1 and E2 were copurified to approx. 90% purity by mild detergent extn., followed by chromatog. on Galanthus nivalus lectin-agarose and DEAE-Fractogel. Immunization of chimpanzees with **purified** E1-E2 generated high titers of anti-E1 and anti-E2 antibodies. Further studies demonstrated that **purified** E1-E2 complexes were recognized at high frequency by **HCV**+ human sera and generated protective immunity in chimpanzees, suggesting that these **purified HCV envelope proteins** display native **HCV** epitopes.

L15 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:164880 HCAPLUS
DOCUMENT NUMBER: 118:164880
TITLE: Expression and identification of hepatitis C virus polyprotein cleavage **products**
AUTHOR(S): Grakoui, Arash; Wychowski, Czeslaw; Lin, Chao; Feinstone, Stephen M.; Rice, Charles M.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110-1093, USA
SOURCE: Journal of Virology (1993), 67(3), 1385-95
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Hepatitis C virus** (**HCV**) is the major cause of transfusion-acquired non-A, non-B hepatitis. **HCV** is an enveloped pos.-sense RNA virus which has been classified as a new genus in the flavivirus family. Like the other two genra in this family, the flaviviruses and the pestiviruses, **HCV** polypeptides appear to be produced by translation of a long open reading frame and subsequent proteolytic processing of this polyprotein. In this study, a cDNA clone encompassing the long open reading frame of the **HCV** H strain (3,011 amino acid residues) has been assembled and sequenced. This clone and various truncated derivs. were used in **vaccinia virus** transient-expression assays to map **HCV**-encoded polypeptides and to study **HCV** polyprotein processing. **HCV** polyproteins and cleavage **products** were identified by using convalescent human sera and a panel of region-specific polyclonal rabbit antisera. Similar results were obtained for several mammalian cell lines examd., including the human HepG2 hepatoma line. The data indicate that at least 9 polypeptides are produced by cleavage of the **HCV** H strain polyprotein. Putative structural proteins, located in the **envelope proteins**, E1 (31 kDa) and E2 (70 kDa), which are heavily modified by N-linked glycosylation. The remainder of the polyprotein probably encodes nonstructural proteins including NS2 (23 kDa), NS3 (70 kDa), NS4A (8 kDa), NS4B (27 kDa), NS5A (58 kDa), and NS5B (68 kDa). An 82- to 88-kDa glycoprotein which reacted with both E2 and NS2-specific **HCV** antisera was also identified (called E2-NS2). Preliminary results suggest that a fraction of E1 is assocd. with E2 and E2-NS2 via disulfide linkages.

L15 ANSWER 15 OF 15 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:528131 HCAPLUS
DOCUMENT NUMBER: 117:128131

TITLE: Hepatitis C virus asialoglycoproteins **manufacture** for **vaccines** or immunoassay
INVENTOR(S): Ralston, Robert O.; Marcus, Frank; Thudium, Kent B.; Gervase, Barbara A.; Hall, John A.
PATENT ASSIGNEE(S): Chiron Corp., USA
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9208734	A1	19920529	WO 1991-US8272	19911107
W: AU, CA, CS, FI, HU, JP, NO, PL, RO, SU				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
EP 414475	A1	19910227	EP 1990-309120	19900821
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 161041	E	19971215	AT 1990-309120	19900821
ES 2110411	T3	19980216	ES 1990-309120	19900821
CA 2064705	AA	19910226	CA 1990-2064705	19900822
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WO 9102820	A1	19910307	WO 1990-US4766	19900822
W: AU, CA, JP				
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RW: BF, BJ, CF, CG, CM, GA, ML, MR, SN, TD, TG				
AU 9176510	A1	19911030	AU 1991-76510	19910329
AU 639560	B2	19930729		
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PL 172133	B1	19970829	PL 1991-296329	19910329
RU 2130969	C1	19990527	RU 1991-5053084	19910329
EP 450931	A1	19911009	EP 1991-302910	19910403
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EP 693687	A1	19960124	EP 1995-114016	19910403
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AT 139343	E	19960615	AT 1991-302910	19910403
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CZ	289923	B6	20020417	CZ 1997-2196	19970710
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PRIORITY APPLN. INFO.:

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EP	1991-302910	A3 19910403
CZ	1993-824	A3 19911107
EP	1992-900091	A3 19911107
JP	1992-500944	A3 19911107
JP	1998-103178	A3 19911107
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US	1992-910760	A3 19920707
FI	1993-2025	A 19930505
US	1993-97853	A1 19930727

AB Two **hepatitis C virus** (**HCV**) **envelope proteins** (E1 and E2) are **manufd.** without sialylation. Expression of these genes in lower eukaryotes, or in mammalian cells in which terminal glycosylation is blocked, results in proteins similar to native **HCV** glycoproteins. When isolated by mannose-binding GNA (Galanthus nivalus agglutinin) lectin affinity, the E1 and E2 proteins aggregate into virus-like particles. Cells bearing a mannose receptor or asialoglycoprotein receptor are capable of being infected with **HCV** and of supporting culturing of the virus. E1 and E2 were produced in HeLa S3 cells inoculated with **recombinant Vaccinia virus** contg. **HCV** gene fragments and **purified** using a GNA-agarose column.